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Stereochemistry of Internucleotide Bond Formation by Polynucleotide Phosphorylase from *Micrococcus luteus*†

Peter M. J. Burgers and Fritz Eckstein*

ABSTRACT: Polynucleotide phosphorylase catalyzes the formation of polynucleotides from the *Sp* diastereomer of adenosine 5′-*O*-(1-thiodiphosphate) (ADPαS), whereas the *Rp* diastereomer is a competitive inhibitor. The absolute configuration of the phosphorothioate diester bond in the polymer was determined by copolymerizing ADPαS,*Sp* isomer with UDP and degrading the resulting copolymer with RNase A and spleen phosphodiesterase to give, inter alia, uridine 2′,3′-cyclic phosphorothioate. The latter product was shown to be the endo isomer by high-performance liquid chromatography. No evidence for the presence of the exo isomer was obtained. It can thus be concluded that the *Sp* diastereomer of ADPαS polymerizes with inversion of configuration at phosphorus without racemization to give a phosphorothioate diester bond with the *Rp* configuration.

Recently, we reported on the use of diastereomeric phosphate analogues for the elucidation of the stereochemical course of enzymatic internucleotide bond formation (Eckstein et al., 1976, 1977). It was found that only one of the two diastereomers of adenosine 5′-*O*-(1-thiotriphosphate), the one arbitrarily designated as isomer A, was accepted as a substrate by *Escherichia coli* DNA-dependent RNA polymerase as well as by Baker's yeast tRNA nucleotidyl transferase. Moreover, in both cases, the internucleotide phosphorothioate diester bond was shown by enzymatic analysis to possess the *R* configuration around the phosphorus atom. Since very recently we also know that the A diastereomer of ATPαS has the *S* configuration at the phosphorus atom (Burgers & Eckstein, 1978). This knowledge, together with our previous results, allows us to determine the complete stereochemistry of action of nucleotidyl transferases.

In this paper we report on the polymerization of adenosine 5′-*O*-(1-thiodiphosphate) (ADPαS,[1] Figure 1) catalyzed by polynucleotide phosphorylase from *Micrococcus luteus* and on the stereochemistry of action of this enzyme.

Materials and Methods

Nucleoside diphosphates were purchased from Waldhof, Mannheim, Germany, and purified over a DEAE-Sephadex column (20 g of DEAE-Sephadex A25/100 mg of product). A linear gradient of 0.05 to 0.4 M triethylammonium bicarbonate (TEAB; 500 mL of each) was applied. [^{14}C]UDP and [^{14}C]ADP were products from Radiochemical Centre, Amersham, and were used without further purification.

ApU, Ap_5A, *M. luteus* polynucleotide phosphorylase (30 units/mg), ribonuclease A, spleen phosphodiesterase (2 units/mg), and alkaline phosphatase (400 units/mg) were obtained from Boehringer, Mannheim, Germany. Rabbit muscle myosin was a gift of Dr. H. Wiedner (Göttingen).

Polygram Cell 300 PEI/UV 254 Fertigfolien from Macherey & Nagel, Düren, Germany, were developed in system A (0.75 M KH_2PO_4, pH 3.5) and DC-Fertigplatten Cellulose F from Merck, Darmstadt, Germany, in system B (1 M NH_4OAc–EtOH, 3:7, v/v).

The high-performance liquid chromatograph employed was a Packard–Becker 8200 chromatograph, equipped with a Packard 1170 UV detector operating at 254 nm and a Servogor RE 511 recorder. The strong anion exchanger Nucleosil 10 SB from Macherey & Nagel was stirred three times with buffer A (0.5 M NH_4OAc, pH 4.4) and decanted to remove the fine particles and then packed into a stainless steel column (40 cm × 2 mm) according to the slurry method. Isocratic elution of the column was normally performed with the same buffer [however, elution of ADPαS (Figure 2) and elution of the products of the nearest-neighbor analysis were effected with buffer B (0.25 M KCl, 0.05 M KH_2PO_4, pH 4.5)]. The flow rate was 1.0 mL/min at pressures of 180–220 atm.

Adenosine 5′-O-(1-Thiodiphosphate). (1) Sp Diastereomer. Rabbit muscle myosin (5 mg/mL; 0.3 mL) was added to an

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[1] Abbreviations used: ADPαS,*Sp* and ADPαS,*Rp*, diastereomers of adenosine 5′-*O*-(1-thiodiphosphate); ATPαS,*Sp*, *Sp* diastereomer of adenosine 5′-*O*-(1-thiotriphosphate); Ap_5A, P^1,P^5-di(adenosine 5′-)-pentaphosphate; Up(S)A, uridyl(3′-5′)adenyl *O*,*O*-phosphorothioate; U>pS, uridine 2′,3′-cyclic *O*,*O*-phosphorothioate; U>p, uridine 2′,3′-cyclic phosphate.

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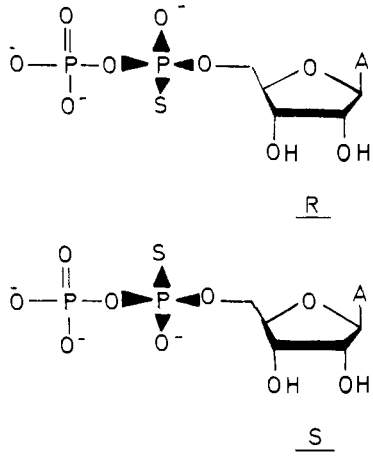

FIGURE 1: Diastereomers of ADPαS.

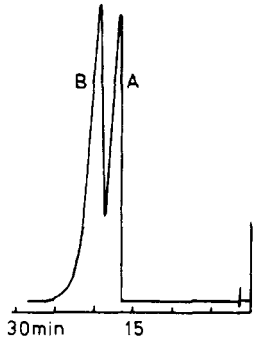

FIGURE 2: LC analysis of the diastereomers of ADPαS: peak A (16.5 min), ADPαS,*Sp*; peak B (19.2 min), ADPαS,*Rp*.

incubation mixture (total volume 41 mL) of 3.66 mM ATPαS (isomer A, *Sp* configuration; enzymatically prepared according to Eckstein & Goody, 1976), 5 mM $CaCl_2$, and 125 mM Tris-HCl (pH 8.0). The reaction was followed by TLC on PEI cellulose plates in system A. After 3 h at 37 °C ca. 50% of the triphosphate had reacted and another 0.3 mL of myosin was added. After 16 h at 37 °C, when the reaction had gone to about 90% completion, the reaction solution was chromatographed on a DEAE-Sephadex A25 column (12 × 2.5 cm) with a linear gradient of 1.5 L each of 0.05 and 0.5 M TEAB. The appropriate fractions were evaporated in vacuo, and the buffer was removed by repeated evaporation with methanol; yield, 1510 A_{260} units (66%). Analysis by high-pressure liquid chromatography (LC) showed this product to be uncontaminated with the *Rp* isomer. Figure 2 shows a separation of a mixture of the diastereomers.

(*2*) *Rp Diastereomer.* This was the unreacted isomer which remained after reaction of the chemically synthesized mixture of diastereomers of ADPαS with pyruvate kinase and phosphoenol pyruvate (Eckstein & Goody, 1976). It was purified by chromatography on DEAE-Sephadex A25 as described above and was shown to be the pure diastereomer by LC.

The labeled [^{35}S]ADPαS diastereomers with a specific activity of 4 mCi/mmol were prepared in essentially the same way.

Polymerization of ADPαS and ADP. The assay solution contained 100 mM Tris-HCl (pH 8.25), 1.0 mM Mn^{2+}, 0.05 mM Ap_5A, 0.05 mM ApU, 600 μg of PNPase/mL, and 2 mM [^{14}C]ADP, [^{35}S]ADPαS,*Sp* isomer, or [^{35}S]ADPαS,*Rp* isomer. The solutions were incubated at 37 °C. At appropriate times, aliquots (20 μL) were taken and applied to Whatman 3 MM filter discs. These were stirred in 10% Cl_3CCOOH and processed for counting of radioactivity as described (Bollum, 1966).

Synthesis of Copolymer of UDP and ADPαS,Sp Diastereomer. The incubation solution contained, in a total volume of 3 mL, 100 mM Tris-HCl (pH 8.25), 4 mM Mn^{2+}, 10 mM [^{35}S]ADPαS,*Sp* diastereomer (353 000 cpm/μmol), 5 mM UDP, 0.1 mM Ap_5A, and 72 units of PNPase. The reaction was followed by counting the radioactivity precipitable in 10% Cl_3CCOOH from 5-μL aliquots which had been applied to Whatman 3 MM discs (Bollum, 1966). After 18 h at 37 °C, another 36 units of PNPase was added, and, after a total reaction time of 44 h, the reaction mixture was diluted with water to 10 mL and the protein extracted with chloroform/isoamyl alcohol (5:2 v/v; 2 × 10 mL). The aqueous solution was concentrated to 2 mL and chromatographed over a Sephadex G-25 column (40 × 2.5 cm). The column was eluted with 0.01 M TEAB buffer. The copolymer eluted in the void volume, well separated from the diphosphates; yield 143 A_{260} units (2 × 10^6 cpm, 18.8% incorporation of ^{35}S label).

Analysis of the Copolymer. (*1*) *Hypochromicity.* The hypochromicity of the copolymer, determined by alkaline degradation (0.5 N KOH, 16 h, 37 °C), was 38%. From this result and the total yield of copolymer (143 A_{260} units) it follows that 229 A_{260} units of mononucleotides was incorporated. From the amount of ^{35}S label incorporated into the copolymer it may be concluded that the latter contains 87 A_{260} units (18.8% of 30 μmol = 5.64 μmol) of adenosine phosphorothioate and, therefore, 142 A_{260} units (14.2 μmol) of uridine phosphate. The ratio U:A in the copolymer was thus 71:29.

(*2*) *Degradation to Nucleosides.* A solution of the copolymer (3 A_{260} units; 42 000 cpm) in aqueous ethanol (15%, 0.3 mL) containing 0.5% $NaHCO_3$ was treated with ethanolic iodine (10 mM, 25 μL) at 0 °C for 10 min to desulfurize the phosphorothioate groups. Excess iodine was repeatedly extracted with ether. Traces of ether were removed by evaporation in vacuo, and an aliquot (100 μL) of the resulting aqueous solution was analyzed for radioactivity in acid-precipitable material. None was observed. The remaining solution (200 μL) was then brought to 5 mM $MgCl_2$, and snake venom PDE (2 μg) and alkaline phosphatase (10 μg) were added. After 1 and 6 h at 37 °C, aliquots (10 μL) were withdrawn and analyzed by LC. Only uridine and adenosine were found to be present in the ratio 68:32, in close agreement with the results obtained from the hypochromicity measurement.

(*3*) *Nearest-Neighbor Analysis.* The copolymer (2 A_{260} units) was hydrolyzed with 0.5 N KOH (50 μL) at 37 °C for 16 h. The mixture was then analyzed by LC (results not shown). The following distribution (percent of total nucleotide material) of products was obtained: U(2′ + 3′)p, 59%; U(2′ + 3′)pS, 12%; A(2′ + 3′)p, 14%; A(2′ + 3′)pS, 15%. The ratio of nucleoside phosphorothioates formed is U(2′ + 3)pS/A(2′ + 3′)pS = 44:56.

Digestion of the Copolymer with RNase A and Spleen Phosphodiesterase. The ^{35}S-labeled copolymer (75 A_{260} units, 1.05 × 10^6 cpm) was dissolved in 20 mL of an incubation mixture containing 10 mM Tris-HCl (pH 7.6) and 4 μg of ribonuclease A. Previous test reactions had shown that under these conditions no acid-precipitable radioactivity remained after 2 min at 25 °C. The solution was kept at room temperature for 10 min and then applied to a DEAE-Sephadex A25 column (4.5 × 2.5 cm). RNase was removed by washing the column with 0.05 M TEAB (150 mL). The nucleotide material was then eluted with 1 M TEAB (200 mL). The high-salt eluant was concentrated to dryness, repeatedly coevaporated with methanol–water (1:1, 3 × 100 mL), and

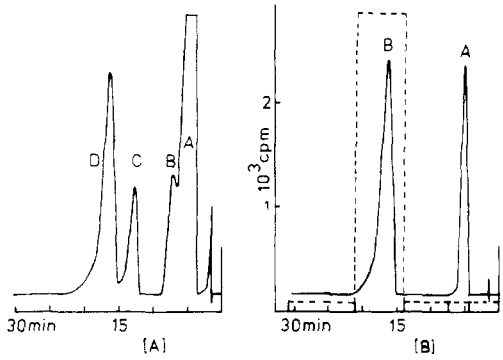

FIGURE 3: LC analysis of digestion products of the copolymer. (A) After DEAE-Sephadex chromatography (fractions 25–35): peak A (3.8–5.5 min), U>p, Up, and unidentified product; peak B (6.8 min), unidentified; peak C (13.0 min), Ap; peak D (16.4 min), U>pS endo isomer. (B) The U>pS-containing fractions (29–37) after second DEAE-Sephadex chromatography: (—) absorbance at 254 nm, peak A (5.0 min), unidentified; peak B (16.4 min), U>pS (endo); (– – –) radioactivity measured from the pooled fractions.

finally dissolved in 20 mL of an incubation solution containing 100 mM NH_4OAc (pH 5.5), 0.1 mM EDTA, 0.05% Tween 80, and 150 μg of spleen phosphodiesterase. After 8 h at 37 °C, the solution was diluted with water to 10 mL and chromatographed over a DEAE-Sephadex A25 column (5 × 2.5 cm) with a linear gradient of 0.05 and 0.25 M TEAB (2 × 300 mL). This gradient eluted mainly mononucleotides, while higher oligonucleotides were retained on the column. Fractions of 6 mL were collected. Fractions 17–22 (10 A_{260} units) contained U>p and no radioactivity. Fractions 25–35 (43 A_{260} units, 324000 cpm) contained U>pS, as shown by LC analysis (Figure 3A), together with U>p, adenosine 3′-phosphate, uridine 3′-phosphate, and other unidentified products. Further fractions contained mainly oligonucleotides with phosphorothioate groups undigested by spleen phosphodiesterase. Fractions 25–35 were collected, concentrated, and then applied to several cellulose F plates. The plates were developed in system B, and the region between R_f 0.5 and 0.8 was scraped off. The nucleotidic material was eluted from the cellulose with 0.5 M TEAB until no more radioactivity eluted. The eluant (263000 cpm) was concentrated to dryness, reevaporated with water–methanol (1:1, 2 × 20 mL), and chromatographed over a second DEAE-Sephadex A25 column (9 × 1 cm) with a linear gradient of 250 mL each of 0.05 and 0.25 M TEAB. Fractions 29–37 contained the desired [^{35}S]U>pS together with an unidentified impurity, which, however, did not contain any radioactivity (Figure 3B). The U>pS solution had a total radioactivity of 24100 cpm, which corresponded to 23% of the radioactivity incorporated into the polymer.

Results

Both diastereomers of ADPαS were tested as possible substrates for polynucleotide phosphorylase from *M. luteus*. Only the *S* isomer was found to be a substrate, with Michaelis constants similar to ADP but with much lower rates of incorporation (Table I), with Mg^{2+} as well as with Mn^{2+} as divalent cation. Linear Lineweaver–Burk plots were obtained for the initial polymerization rates of ADP with Mg^{2+} and Mn^{2+} at the constant ratio $[ADP]/[Me^{2+}] = 2$. Linear plots were also obtained for ADPαS isomer *S* at low (<5 mM) substrate concentrations and $[ADP\alpha S]/[Me^{2+}] = 2$. At much higher concentrations of ADPαS isomer *S*, substrate inhibition was observed. Both diastereomers of ADPαS were competitive inhibitors of the polymerization of ADP (Table I). In the kinetic studies the primer ApU (50 μM) was added to eliminate the lag phase occurring in unprimed PNPase-catalyzed polymerizations (Godefroy et al., 1970). Addition of this primer resulted in linear initial rates of polymerization.

Table I: Kinetic Parameters of ADP and ADPαS[a]

	Mg^{2+}			Mn^{2+}	
divalent ion	K_m (mM)	V_{max}[b]	K_i[c] (mM)	K_m (mM)	V_{max}[b]
ADP	2.8	320		0.9	60
ADPαS,*S*p	3.8	1.1	1.0	0.6	1
ADPαS,*R*p		<0.02	1.6		<0.02

[a] Experimental conditions as described in Figure 4 and in Results. [b] In nanomoles per minute; normalized to V_{max} (ADPαS,*S*p, Mn^{2+}) = 1 nmol/min. [c] Apparent inhibitor constants for the polymerization of ADP.

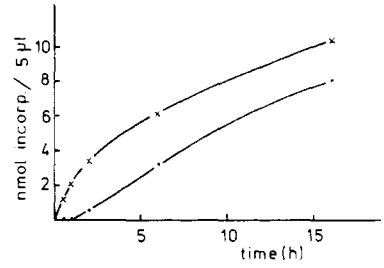

FIGURE 4: Copolymerization of UDP and ADPαS,*S*p isomer. The assay solution contained 100 mM Tris-HCl at pH 8.25, 4 mM Mn^{2+}, 0.1 mM Ap_5A, 900 μg of PNPase/mL, 10 mM ADPαS,*S*p isomer, and 5 mM UDP. In one experiment (x–x) [^{14}C]UDP and in the second (●–●) [^{35}S]ADPαS,*S*p isomer were used as radioactive tracers. The solutions were incubated at 37 °C and, after the times indicated, 5-μL aliquots were removed, and the acid-precipitable radioactivity was determined on Whatman 3 MM discs.

Because the commercial PNPase contained a myokinase activity, as shown by the conversion of ADPαS to adenosine 5′-*O*-phosphorothioate and ATPαS, Ap_5A was added to all incubation solutions (Lienhard & Secemski, 1973). A concentration of 50 μM was sufficient to suppress the myokinase activity completely.

Preliminary tests showed that the copolymerization of UDP and ADPαS isomer *S* was best performed with $[ADP\alpha S]/[UDP] = 2$ (Figure 4). Under these conditions an equal rate of incorporation of both nucleosides occurred for at least half of the total incorporation of adenosine, thus ensuring that phosphodiester bonds with the necessary sequence Up(S)A would be formed. Limited RNase treatment of the copolymer, followed by spleen phosphodiesterase treatment, led to the formation of U>pS.

The ribonuclease reaction was carried out for such a long time that also internucleotide Up(S)A linkages with the *S*p configuration, if present, would have been transesterified to U>pS.

After initial purification over DEAE-Sephadex, U>pS was isolated together with mononucleotides. LC analysis (Figure 3a) showed a peak at the retention time (15.4 min) of U>pS (endo), a second peak at 13.0 min close to the retention time of U>pS (exo) (13.5 min), but assigned to adenosine 3′-phosphate, and other peaks with lower retention times. That the presence of this second peak was not due to U>pS (exo) was shown by treatment of a small sample with alkaline phosphatase. The first peak at 16.4 min remained while the other peaks, including the second at 13.0 min, had shifted to much lower retention times (results not shown). Chromatography on cellulose plates very efficiently separated the nucleoside monophosphates (R_f 0.1) from U>pS (R_f 0.6).

Finally, chromatography for a second time over DEAE-Sephadex allowed us to isolate U>pS (endo isomer), con-

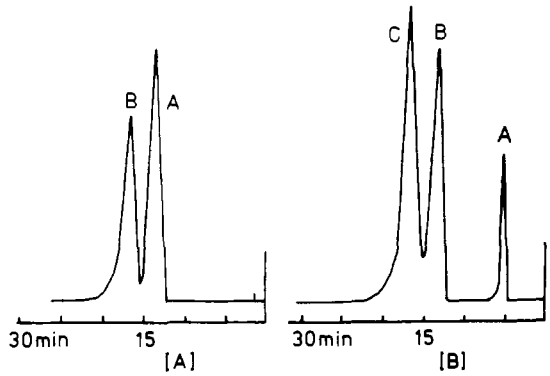

FIGURE 5: LC analysis of purified U>pS from the enzymic digest. (A) Separation of authentic endo and exo isomers of U>pS; peak A (13.4 min), exo isomer; peak B (16.3 min), endo isomer. (B) Admixture of pure U>pS exo isomer to the U>pS containing fraction after second DEAE-Sephadex chromatography; peak A (5.2 min), unidentified; peak B (13.6 min), U>pS (exo); peak C (16.4 min), U>pS (endo).

taminated with one other unidentified product, but with all radioactivity coinciding with the U>pS peak (Figure 3B).

Comparison by LC analysis of the above U>pS solution with authentic U>pS (endo isomer) and U>pS (exo isomer) showed that the U>pS formed by enzymatic digestion of the copolymer was the endo isomer (Figures 3B and 5A). When authentic endo isomer was added to the product the LC analysis was as in Figure 3B, whereas when authentic exo isomer was added the pattern shown in Figure 5B was obtained.

A further proof that the product isolated from the enzymatic treatment of the copolymer is indeed U>pS was obtained by desulfurization with iodine. This reaction was performed under the same conditions as described above for the desulfurization of the copolymer. A reaction product was obtained with a retention time identical with that of U>p (results not shown).

Discussion

We had shown earlier that a mixture of the diastereomers of uridine 5′-*O*-(1-thiodiphosphate) is a substrate for polynucleotide phosphorylase (Gindl & Eckstein, 1970). However, it is now possible to separate the stereoisomers of ADPαS by enzymatic methods (Eckstein & Goody, 1976). Both diastereomers of ADPαS are competitive inhibitors for the polymerization of ADP, while only the *S*p isomer is accepted as a substrate. This shows that ADPαS occupies the same binding site on the enzyme as the normal nucleoside diphosphates. Furthermore, the similar K_m values obtained for ADP and ADPαS, isomer *S* in the Mg–PNPase as well as the Mn–PNPase system, indicate that the mechanism and the stereochemistry of action of PNPase with ADPαS, isomer *S*, as substrate will also hold for the normal nucleoside diphosphates.

The determination of the configuration of the internucleotide phosphorothioate diester bond formed is as yet not possible by direct measurement of the polymer, and thus use was made of the known stereochemical course of transesterification of uridine 2′,3′-cyclic phosphorothioate catalyzed by RNase A. The enzymatic transesterification reactions of U>pS with methanol (Saenger et al., 1974) and cytidine (Usher et al., 1972) have been studied. It was shown that the endo isomer of U>pS produces a phosphorothioate diester linkage with the *R* configuration and that, therefore, this reaction proceeds via an in-line mechanism.

On the basis of these results we decided to prepare random copolymers of uridine and adenosine containing the Up(S)A

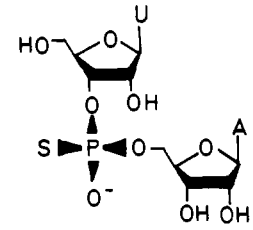

FIGURE 6: Internucleotide phosphorothioate group in *R* configuration.

bond. These bonds can be transesterified by RNase A to the cyclic phosphorothioates which can be isolated before they are cleaved further to the phosphorothioates. The configuration of the cyclic phosphorothioate can then be determined (Eckstein et al., 1976).

In Figure 4 it can be seen that, after an initial rapid homopolymerization of uridine, an approximately 1:1 copolymerization of uridine and adenosine took place. From the nearest-neighbor analysis of the copolymer we know that 44% of the internucleotide phosphorothioate linkages are Up(S)A linkages and 56% Ap(S)A linkages. Upon degradation of the polymer with RNase A only the former type of internucleotide bonds would hydrolyze to (oligo) nucleotides with a terminal U>pS moiety. Subsequent treatment with spleen phosphodiesterase would then hydrolyze the remaining internucleotide phosphodiester bonds and produce U>pS.

The identification of the latter product posed a problem. The yield was too low to isolate the material by crystallization and compare the crystalline product with authentic material by NMR spectroscopy and melting point as in the experiments with RNA polymerase (Eckstein et al., 1976). For the detection and identification of the product we have in the present study relied entirely on chromatographic methods. The diastereomers of U>pS are easily separated by LC with retention times of 13.4 and 16.3 min for the exo and endo isomer, respectively (Figure 5A). After degradation of the copolymer by RNase and spleen PDE, the entire purification procedure was monitored by LC as described under Results. The material finally isolated consisted of two products, of which only the one identified as U>pS endo isomer contained radioactivity (Figure 3B). The yield of isolated U>pS was 52%, based on the fraction of Up(S)A linkages in the polymer. This rather low yield can be ascribed to losses during the many chromatographic steps necessary for its purification and to a possible further enzymatic breakdown of the cyclic phosphorothioate to the monophorothioate during the RNase treatment. The probability that U>pS exo isomer would have been lost during these purification steps can be excluded because the diastereomers cannot be separated in the systems used.

The identification of the isolated product as U>pS endo isomer comes from comparison by LC analysis of this material with authentic endo and exo isomer (see under Results). From the results with RNase A it is known that the endo isomer of U>pS is produced by transesterification of an internucleotide phosphorothioate linkage with the *R* configuration.

We can thus conclude that the *S*p diastereomer of ADPαS is polymerized by polynucleotide phosphorylase from *M. luteus* to give a polymer with phosphorothioate linkages in the *R* configuration (Figure 6). The polymerization thus proceeds by inversion of configuration at the phosphorus atom.

The results obtained in this study are thus identical with those obtained earlier with DNA dependent RNA polymerase and tRNA nucleotidyl transferase. All three enzymes accept the same configurational diastereomer (*S*p) of ADPαS or ATPαS, respectively, as substrate, while the *R*p diastereomer

is a competitive inhibitor. Furthermore, the internucleotide phosphorothiate bond formed always has the *R* configuration. It seems likely that the inversion of configuration is the result of a simple in-line mechanism where in the transition state the incoming 3′-hydroxy function as well as the leaving phosphate or pyrophosphate group occupies the apical positions of a trigonal bipyramid (Eckstein et al., 1976; Burgers & Eckstein, 1978).

Acknowledgments

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Noninteger Pitch and Nuclease Sensitivity of Chromatin DNA†

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ABSTRACT: Assuming that variation of nuclease sensitivity along nucleosomal DNA can basically be attributed to orientations of sugar–phosphate bonds relative to histone core, the pitch of chromatin DNA is estimated to be 10.33–10.40 base pairs. This is in accordance both with the known measured average distance between cleavage sites (10.3–10.4 base pairs) and with published data on variation of relative sensitivities of these sites to nuclease attack. The variation can be explained solely as a result of the systematic change of orientation of sugar–phosphate bonds of sensitive sites without additional suggestions about local steric hindrances by histone molecules. According to the analysis locations of sites least sensitive to nuclease attack should not depend on kind of endonuclease though the stagger could differ. We conclude that the nucleosome core particle is axially symmetrical. The results strongly support the suggestion that DNA is wrapped around the histone octamer smoothly, without interruption of base-stacking interactions.

An important structural feature of chromatin is the limited sensitivity of its DNA to nuclease digestion which is periodically varying along the DNA with a period equal or close to ten base pairs (Noll, 1974, 1977). One suggested explanation of the phenomenon is that the periodical distribution of sensitive sites along the chromatin DNA results from periodical variation of exposure of bonds to the surroundings due to the helical structure of chromatin DNA (Noll, 1974, 1977). According to the nuclease digestion data and to low angle neutron scattering data (Pardon et al., 1975), DNA in the nucleosome is folded around the histone core so that only the outside surface of DNA is accessible to nucleases. The regularity of digestion has led to the suggestion that the folding of DNA is smooth, without disruption of base stacking interactions (Noll, 1974, 1977). The possibility of such smooth folding of DNA was demonstrated recently both from energetical and stereochemical points of view (Sussman & Trifonov, 1978; Levitt, 1978) and supported experimentally (Kallenbach et al., 1978).

An alternative explanation of the periodical variation of nuclease sensitivity of chromatin DNA is a kinked helix (Crick & Klug, 1975) with kinks repeating every ten base pairs.

Recent measurements of lengths of nuclease digestion fragments of nucleosomal DNA using sequenced standards led to the conclusion that the average distance expressed in base pairs between adjacent sensitive sites in chromatin DNA is noninteger: 10.3–10.4 (Prunell et al., 1979). This finding is also supported by recent accurate length determinations of nucleosomal DNA (Tatchell & Van Holde, 1978; Seligy & Poon, 1978) which is found to be 145 rather than 140 base pairs. It leads to the same figure as above.

If the nuclease sensitivity of nucleosomal DNA is determined basically by orientation of base pairs relative to the histone core, then this noninteger number of base pairs should correspond to the pitch of chromatin DNA. An important consequence of nonintegerness of the pitch of DNA is variation of orientations of sugar–phosphate bonds to be disrupted. It should lead to a kind of beating effect, i.e., modulation of periodical sensitivity of nucleosomal DNA. Such modulation was demonstrated indeed by analysis of fragments of 5′-^{32}P-labeled nucleosomal DNA, obtained by nuclease digestion of nucleosomes. Endonucleases tested were of different structure, active in different optimal conditions: DNase I (Simpson & Whitlock, 1976; Whitlock et al., 1977; Noll, 1977; Simpson, 1978), endonuclease of *Aspergillus oryzae*, and DNase II (Whitlock et al., 1977). It was found that sites of nucleosomal DNA positioned at about 30, 110, and, less pronounced, 60 and 80 base pairs from the ends of the DNA are less sensitive to nuclease digestion than, correspondingly, at other multiples of 10 or so base pairs. The variation (modulation) of the sensitivity was ascribed to protection provided by local steric hindrances by histones (Whitlock et al., 1977; Finch et al., 1977).

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